

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout (*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

**Data Requirement:**

PMRA DATA CODE	{.....}
EPA DP Barcode	D290348
OECD Data Point	
EPA MRID	45826703
EPA Guideline	72-1(c)

**Test material:** BAS 500 00F (Headline Fungicide)**Purity:** 247.83 g/L a.i.


Common name: Pyraclostrobin

Chemical name: IUPAC: Not reported

CAS name: Not reported

CAS No.: Not reported

Synonyms: Not reported

**Primary Reviewer:** Rebecca Bryan  
Staff Scientist, Dynamac Corporation**Signature:****Date:** 3/1/04**QC Reviewer:** Gregory Hess  
Staff Scientist, Dynamac Corporation**Signature:****Date:** 3/1/04**Approved By:** Lewis Ross Brown, Biologist  
OPP/EFED/ERB - **Date:** 02/10/05

02/10/05

**Reference/Submission No.:****Company Code:****Active Code:****EPA PC Code:** 099100**Date Evaluation Completed:**

**CITATION:** Zok, S. 1999. BAS 500 00F (Headline Fungicide)- Acute Toxicity Study on the Rainbow Trout (*Oncorhynchus mykiss* WALBAUM 1792) in a Static System (96 hours). Unpublished study performed by BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project Identification No. 12F0185/975052 (BASF Reg. Doc. No. 1999/11211). Study sponsored by BASF-Aktiengesellschaft; D-67056 Ludwigshafen, Federal Republic of Germany. Study submitted by BASF Corporation, Agricultural Products, Research Triangle Park, NC. Experimental start date September 21, 1998 and experimental termination date September 25, 1998. The final report issued August 30, 1999.



2022945

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout (*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

**EXECUTIVE SUMMARY:**

In a 96-hour acute toxicity study, Rainbow trout (*Oncorhynchus mykiss*) were exposed to BAS 500 00F (Headline Fungicide, a.i. Pyraclostrobin) at nominal concentrations of 0.0 (negative control), 0.0100, 0.0147, 0.0215, 0.0316, 0.0464, 0.0681 and 0.1000 ppm under static conditions. Mean-measured concentrations (1 plus 96 hours) were < LOQ (control), 0.0081, 0.0123, 0.0182 and 0.0274 ppm. Mean measured values for the nominal 0.0464, 0.0681 and 0.1000 ppm treatment groups were not reported.

After 96 hours of exposure, 70, 100, 100, 100, and 100% mortality was observed in the nominal 0.0215, 0.0316, 0.0464, 0.0681, and 0.1000 ppm treatment groups, respectively. No mortalities were observed in the control, 0.0100 or 0.0147 ppm treatment groups. The sub-lethal effects included swimming near the bottom in the nominal 0.0215 ppm treatment group after 72 and 96 hours, fish swimming near the bottom, apathy, and/or discoloration in the 0.0316 ppm treatment group after 24, 48 and 72 hours and convulsions in the 0.0464 ppm treatment group after 24 hours. Convulsions and swimming near the bottom were also observed in the 0.0681 ppm treatment group after 4 hours, while a narcotic-like state was observed in the 0.1000 ppm treatment group after 4 hours.

The 96 hour recovery for the measured 0.009874 (67.2%) ppm treatment group was less than 70% of the nominal 0.0147 ppm treatment group, and the 0.0464, 0.0681 and 0.1000 ppm nominal test concentrations were not measured at 96 hours. Consequently, this study is classified as **INVALID**. This study is not scientifically sound because the actual concentrations which fish were exposed to are unknown. The test material declined to an unacceptable level, reportedly because the test compound shows a high affinity to glass surfaces, and it may have adhered to the test chambers (p. 44). The study author provided no further details regarding the poor solubility, nor made mention that attempts were made to improve test material recovery after 96 hours. This study does not satisfy the guideline requirement for an acute toxicity study with freshwater fish (Subdivision E, §72-1).

**Results Synopsis**

Test Organism Size/Age (mean Weight or Length): 2.8 g; 6.1 cm  
Test Type (Flow-through, Static, Static Renewal): Static

**96-Hour; Invalid, study-results not reported**

LC<sub>50</sub>: 95% C.I.:

NOAEC:

LOAEC:

Endpoints affected:

Most sensitive endpoint:

**I. MATERIALS AND METHODS**

**GUIDELINES FOLLOWED:**

The study protocol was based on procedures outlined in the EPA guideline, Pesticide Assessment Guidelines, subdivision E, Hazard Evaluation Wildlife and Aquatic Organisms, U.S. Environmental Protection Agency, Washington DC, para. 72-1 (1982); EPA-SEP (Standard Evaluation Procedure) No. 540/9-85-006 (1985); EEC directive 84/449, C.1; and OECD Guideline No. 203, "Fish, Acute Toxicity Test" (1992). Deviations from U.S. EPA guidelines §72-1c included:

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

1. The dilution water hardness (250 mg CaCO<sub>3</sub>/L) was five times higher than recommended (40-48 mg CaCO<sub>3</sub>/L). The pH range (8.2-8.5) was greater than recommended (7.2-7.6).
2. The 96 hour recovery for the measured 0.009874 ppm (67.2%) treatment group was less than 70% of the nominal 0.0147 ppm treatment group.
3. The 0.0464, 0.0681 and 0.1000 ppm nominal test concentrations were not measured at 96 hours.

Failure to accurately measure the test concentrations that fish were exposed to greatly affected the validity and acceptability of the study.

**COMPLIANCE:**

Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. The test was conducted in accordance with the GLP provisions of the "Chemicals Act" (Chemikaliengesetz, Germany) and the OECD Principles of Good Laboratory Practice (Paris, 1981), however, it meets the requirements of 40 CFR Part 160.

**A. MATERIALS:**

1. **Test Material** BAS 500 00 F (Headline Fungicide)

**Description:** Liquid

**Lot No./Batch No. :** 97-2

**Purity:** 247.83 g/L a.i.

**Stability of Compound**

**Under Test Conditions:** The stability of the test substance in the dilution water during the course of the study was verified by analytical determination at 0 (90.1-100.5% of nominal) and 96 hours (67.2-75.6%\*; Table 9.4, p. 24).

\* The 96 hour mean measured concentrations were not reported for the 0.0464, 0.0681 and 0.1000 ppm nominal test concentrations.

*OECD requires water solubility, stability in water and light,  $pK_a$ ,  $P_{ow}$ , and vapor pressure of the test compound. All OECD requirements were not reported.*

**Storage conditions of  
test chemical:**

Stored at room temperature.

2. **Test organism:**

**Species:** Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792)

**Age at test initiation:** Approximately 7 months

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout (*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

**Weight at test initiation:** 2.8 g (2.0-3.6 g)

**Length at test initiation:** 6.1 cm (5.4-6.8 cm)

**Source:** Forellenhof Fredelsloh, Moringen, Germany

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a) Range-finding Study: A non-GLP range-finding study was conducted. The  $LC_{50}$  after 96 hours was between 0.01 and 0.1 mg/L. The definitive nominal test concentrations were based on the range-finder results.

b) Definitive Study:

**Table 1 . Experimental Parameters**

Parameter	Details	Remarks
		Criteria
Acclimation period:	Approximately 2 months.	EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.
Conditions: (same as test or not)	Same as test	
Feeding:	Growing feed (Kronen Fisch Aminostart I estr.), <i>ad libitum</i> , with frozen brine shrimp (artemia) was provided on acclimation workdays, except the last day prior to and during testing.	
Health: (any mortality observed)	0.5% mortality during the last 7 days of acclimation.	
Duration of the test	96 hours	EPA/OECD requires: 96 hours

(*Oncorhynchus mykiss*)

EPA MRID Number 45826703

[illegible]

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

Parameter	Details	Remarks
		Criteria
Source of dilution water	The dilution water was non-chlorinated, charcoal-filtered and aerated, tap water.	<i>EPA 1975; Soft reconstituted water or water from a natural source, <b>not</b> dechlorinated tap water; OECD permits dechlorinated tap water.</i>
<u>Water parameters:</u> Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	250 mg CaCO <sub>3</sub> /L 8.3-8.6 8.7-12.8 mg/L (≥60% saturation) Not reported Not reported 10 mg Mg/L Not reported Not reported 11-12°C (continuous measurements: 10.6-11.1°C) N/A DO, pH, and temperature were determined daily in replicates with surviving fish. Additionally, the temperature was measured hourly in one test aquaria (nominal, 0.0316 ppm treatment group).	The hardness (250 mg/L as CaCO <sub>3</sub> ) was 5x higher than recommended (40-48 mg/L as CaCO <sub>3</sub> ). The pH range (8.3-8.6) was greater than recommended (7.2-7.6).  The test water was regularly assayed for chemical contaminants and microbes.

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

Parameter	Details	Remarks
		Criteria
		<p><b>Hardness and pH</b> EPA requires hardness of 40-48 mg/L as CaCO<sub>3</sub> and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range &lt;0.8. OECD allows hardness of 10-250 mg/L as CaCO<sub>3</sub> and pH between 6 and 8.5.</p> <p><b>Dissolved Oxygen</b> <u>Renewal</u>: ≥60% during 1<sup>st</sup> 48 hrs and ≥40% during 2<sup>nd</sup> 48 hrs <u>Flow-through</u>: ≥60% through out test. OECD requires at least 80% saturation value.</p> <p><b>Temperature</b> EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.</p> <p><b>Salinity</b> 30-34 ‰ (parts per thousand) salinity, weekly range &lt; 6 ‰</p> <p><b>EPA water quality</b> measured at beginning of test and every 48 hours</p>

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout (*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

Parameter	Details	Remarks
		Criteria
<u>Concentration of test material:</u> nominal:  measured:	0 (negative control), 0.0100, 0.0147, 0.0215, 0.0316, 0.0464, 0.0681, and 0.1000 ppm.  ND (not detected, negative control), 0.009, 0.0148, 0.0207, 0.0309, 0.0437, 0.0636, and 0.0961 ppm.	The measured concentrations are the 1-hour mean measured concentrations. The 96-hour measured concentrations were 67.2 - 75.6% of nominal for the 0.007098, 0.009874, 0.015731, and 0.023882 ppm treatment groups. Due to a lack of a 96-hour measured concentration at the nominal 0.0464, 0.0681 and 0.1000 treatment levels (0.058 ppm nominal), the reviewer was unable to calculate a overall mean measured value.  <i>EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series</i>
Solvent (type, percentage, if used)	N/A	<i>EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.</i>
<u>Number of fish/replicates:</u> negative control:  solvent control:  treated:	10 fish, in one test vessel  N/A  10 fish, in one test vessel	<i>EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration</i>
Biomass loading rate	approx. 0.3 g fish/L	<i>Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at &gt; 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through</i>
Lighting	16-hours light/8-hours dark.	



**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout (*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

Parameter	Details	Remarks
		Criteria
		<i>EPA requires: 16 hours light/8 hours dark; OECD requires 12-16 hours photoperiod.</i>
Feeding	Animals were not fed one day prior to and during testing.	<i>EPA/OECD requires: No feeding during the study</i>
Recovery of chemical	67.2-100.5%	Based on analytical recoveries from the 0 and 96 hour samples. The 96 hour recovery for the 0.0147 ppm treatment group was 67.2% of nominal. This was the only recovery less than the recommended 70% of nominal. 96-hour mean measured concentrations were not reported for the 0.0464, 0.0681 and 0.1000 ppm nominal treatment levels.
Level of Quantitation	0.008 mg/L	
Level of Detection	Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

**2. Observations:**

**Table 2: Observations**

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	Every 24 hours.	<i>EPA/OECD requires: minimally every 24 hours</i>
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

**II. RESULTS AND DISCUSSION:**

**A. MORTALITY:**

After 96 hours of exposure, 70, 100, 100, 100, and 100% mortality was observed in the nominal 0.0215, 0.0316, 0.0464, 0.0681, and 0.1000 ppm treatment groups, respectively. No mortalities were observed in the control, 0.0100 or 0.0147 ppm treatment groups.

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)

PMRA Submission Number {.....}

EPA MRID Number 45826703

Table 3: Effect of BAS 500 00F (Headline Fungicide) on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment, ppm, measured <sup>a</sup> and (nominal conc.)	No. of fish at start of study	Observation Period									
		0-4 Hours		24 Hours		48 Hours		72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	10	0	0	0	0	0	0	0	0	0	0
0.0090 (0.0100)	10	0	0	0	0	0	0	0	0	0	0
0.0148 (0.0147)	10	0	0	0	0	0	0	0	0	0	0
0.0207 (0.0215)	10	0	0	0	0	0	0	3	30	7	70
0.0309 (0.0316)	10	0	0	0	0	2	20	7	70	10	100
0.0437 (0.0464)	10	0	0	7	70	10	100	10	100	10	100
0.0636 (0.0681)	10	0	0	10	100	10	100	10	100	10	100
0.0961 (0.1000)	10	10	100	10	100	10	100	10	100	10	100
NOAEC (mortality) <sup>b</sup>	0.0123 ppm										
LC <sub>50</sub> (95% C.I.) <sup>b</sup>	0.0169 ppm (95% C.I. not reported)										
Positive control, if used mortality: LC <sub>50</sub> :	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

<sup>a</sup> The mean measured concentrations are based on the study reported 1-hour results.

<sup>b</sup> The NOAEC and LC<sub>50</sub> were reported by the study author based upon the 1 plus 96-hour mean measured concentrations (p. 33).

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

**B. NON-LETHAL TOXICITY ENDPOINTS:**

The sublethal effects included swimming near the bottom in the nominal 0.0215 ppm treatment group after 72 and 96 hours, fish swimming near the bottom, apathy, and/or discoloration in the 0.0316 ppm treatment group after 24, 48 and 72 hours and convulsions in the 0.0464 ppm treatment group after 24 hours. Convulsions and swimming near the bottom were observed in the 0.0681 ppm treatment group after 4 hours, while a narcotic-like state was observed in the 0.1000 ppm treatment group after 4 hours.

**Table 4. Sublethal effects of BAS 500 00F (Headline Fungicide) on Rainbow trout (*Oncorhynchus mykiss* ).**

Treatment, ppm, measured <sup>c</sup> and (nominal conc.)	Observation Period				
	endpoint at 1-4 Hours	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected <sup>a</sup>	% affected <sup>a</sup>	% affected <sup>a</sup>	% affected <sup>a</sup>	% affected <sup>a</sup>
Negative control	AN	AN	AN	AN	AN
0.0090 (0.0100)	AN	AN	AN	AN	AN
0.0148 (0.0147)	AN	AN	AN	AN	AN
0.0207 (0.0215)	AN	AN	AN	Swimming near the bottom - 70%	Swimming near the bottom - 30%
0.0309 (0.0316)	AN	Swimming near the bottom - 40%	Apathy - 80% Swimming near the bottom - 70% Discoloration - 80%	Swimming near the bottom - 30% Discoloration - 30%	--
0.0437 (0.0464)	AN	Convulsions - 30%	--	--	--
0.0636 (0.0681)	Convulsions - 10% Swimming near the bottom - 30%	--	--	--	--

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

Treatment, ppm, measured <sup>c</sup> and (nominal conc.)	Observation Period				
	endpoint at 1-4 Hours	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected <sup>a</sup>	% affected <sup>a</sup>	% affected <sup>a</sup>	% affected <sup>a</sup>	% affected <sup>a</sup>
0.0961 (0.1000)	Narcotic-like state - 100%	--	--	--	--
NOAEC (sub-lethal) <sup>d</sup>	0.0123 ppm				
LOAEC (sub-lethal) <sup>d</sup>	0.0182 ppm				
EC <sub>50</sub>	Not determined				
Positive control, if used % sub-lethal effect: EC <sub>50</sub> :	N/A <sup>e</sup>	N/A	N/A	N/A	N/A

<sup>a</sup> The % affected was reviewer-calculated based on number affected divided by the number of surviving fish.

-- Not determined due to 100% mortality.

<sup>b</sup> AN - Appeared Normal

<sup>c</sup> The mean measured concentrations are based on the study reported 1-hour results.

<sup>d</sup> The NOAEC and LC<sub>50</sub> were reported by the study author based upon the 1 plus 96 hour mean measured concentrations (p. 13, 21 and 33).

<sup>e</sup> N/A - Not Applicable

### **C. REPORTED STATISTICS:**

The 96-hour LC<sub>50</sub> value was estimated, if possible, using probit analysis at the 1 and 5% significance levels (p. 18 of the study report). The NOAEC was determined based on mortality and sublethal effects data. The LC<sub>50</sub>, NOAEC and LOAEC results were based on the 1 plus 96-hour mean measured concentrations (p. 13, 33), i.e. the mean values calculated from the analytically detected concentrations reported at 1 hour and 96 hours.

LC<sub>50</sub>: 0.0169 ppm                      95% C.I.: Not reported  
NOAEC: 0.0123 ppm  
LOAEC: 0.0182 ppm  
Endpoints affected: Mortality and sub-lethal effects  
Most sensitive endpoint: Mortality and sub-lethal effects

### **D. VERIFICATION OF STATISTICAL RESULTS:**

The LC<sub>50</sub> was determined using the binomial method via TOXANAL statistical software. The NOEC and LOEC were determined using Fisher's Exact test via TOXSTAT software. The LC<sub>50</sub>, NOEC and LOEC values were calculated using the mean measured concentrations from the 1-hour and 96-hour analytical results for the nominal 0.0100, 0.0147, 0.0215 and 0.0316 ppm treatment levels. The reviewer was unable to calculate a mean measured value for the three (3) highest nominal treatment groups, 0.0464, 0.0681 and 0.1000 ppm, because a 96-hour analytical value was not reported; furthermore, because there was total mortality in these groups and the one preceding them, the three highest treatment groups were excluded from the analysis. Results of the reviewer's statistical verification (below) were not reported in the Executive Summary or Conclusion section of this DER because the study is considered **INVALID**.

#### **96-Hour**

LC<sub>50</sub>: 0.0166 ppm                      95% C.I.: 0.0123 to 0.0274 ppm  
NOAEC: 0.0123 ppm  
LOAEC: 0.0182 ppm  
Endpoints affected: Mortality and sub-lethal effects  
Most sensitive endpoint: Sub-lethal effects

### **E. STUDY DEFICIENCIES:**

The nominal 0.0464, 0.0681 and 0.1000 ppm treatment groups were not measured at 96 hours. Furthermore, the measured 96-hour recovery (0.009874 ppm) was less than 70% of nominal. According to the US EPA Pesticide Reregistration Rejection Rate Analysis document (EPA 738-R-94-035, December 1994), a study may be rejected if:

"Not all test solutions were measured at 96 hours as well as at zero hours" (p. 82). "A test material is considered to be stable under test conditions if, under those conditions, it does not degrade, volatilize, dissipate, precipitate, sorb to test container walls, or otherwise decline to concentrations less than 70% of the day-0 measured concentration during the study period. If it is expected to decline to less than 70% of the day-0 measured concentration during the study period, either static renewal or flow-through design is needed to try to ensure that the test concentration is maintained at levels greater than or equal to 70%. The only exception is testing with algae and diatoms, which cannot be tested in static renewal or flow-through systems" (from "Conducting Acceptable Aquatic Lab Studies: Proposed Guidance, 1992, p.4; EPA 738-R-94-035, December 1994).

The study author does not address these solubility issues and there is no report that attempts were made to improve the test material recovery. It is unclear what concentrations fish were actually exposed to, so this study

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

is classified as **INVALID** and it does not fulfill the guideline requirement (Subdivision E, §72-1) for an acute toxicity study with freshwater fish.

**F. REVIEWER'S COMMENTS:**

The mean measured 96-hour test concentration was less than 70% of the nominal and mean measured 1-hour test concentrations within one of the treatment groups. The study is also considered **INVALID** because the study author failed to report a mean measured values for the nominal 0.0464, 0.0681 and 0.1000 ppm treatment group at 96 hours.

The study author-reported "1 plus 96 hours" analytical concentrations (p. 33) represent the arithmetic mean of the 1-hour and 96-hour measured analytical values. The study author excluded the three highest treatment levels (0.0464, 0.0681 and 0.1000 ppm, nominal) from all toxicity calculations (most likely because of the 100% mortality rate by 48 hours). The study author reported within the analytical methods section of the study report (p. 44), that:

"Probably the low analytical results are caused by the physical properties of the active ingredient. Reg.No. 304428 is difficult to dissolve in water without using any additional solvents. Furthermore, it shows a significant adhesion capacity to glass surfaces. The addition of organic solvents to the samples reduces the influence of the surface effect and improves the solubility of the active ingredient."

Results of the reviewer's statistical verification differed slightly from those reported by the study author. The reviewer determined the  $LC_{50}$  value to be 0.0166 ppm (95% C.I.: 0.0123 to 0.0274 ppm), whereas the study author reported the value to be 0.0169 ppm (95% C.I.: not reported). The study author-reported NOAEC and LOAEC values were identical to those determined in the reviewer's statistical verification. The slight difference observed in the  $LC_{50}$  value is presumably due to the different statistical methods used.

**G. CONCLUSIONS:**

The 96 hour recovery for the measured 0.009874 (67.2%) ppm treatment group was less than 70% of the nominal 0.0147 ppm treatment group, and the 0.00.0464, 0.0681 and 0.1000 ppm nominal test concentrations were not measured at 96 hours. Consequently, this study is classified as **INVALID**. This study is not scientifically sound because the actual concentrations which fish were exposed to are unknown. The test material declined to an unacceptable level, reportedly because the test compound shows a high affinity to glass surfaces, and it may have adhered to the test chambers (p. 44). The study author provided no further details regarding the poor solubility, nor made mention that attempts were made to improve test material recovery after 96 hours. This study does not satisfy the guideline requirement for an acute toxicity study with freshwater fish (Subdivision E, §72-1).

**96-Hour; Invalid, study-results not reported**

$LC_{50}$ : 95% C.I.:

NOAEC:

LOAEC:

Endpoints affected:

Most sensitive endpoint:

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

**III. REFERENCES:**

Finney, D.J. 1971. "Probit Analysis". Cambridge University Press, 3<sup>rd</sup> ed.



**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Rainbow trout  
(*Oncorhynchus mykiss*)**

PMRA Submission Number {.....}

EPA MRID Number 45826703

**APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

TOXANAL RESULTS: Calculated using the 1 plus 96-hour mean measured concentrations (p. 33 of the study report). Note, these values represent the overall mean measured concentrations as reported by the study author and verified by the reviewer.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.0274	10	10	100	9.765625E-02
.0182	10	7	70	17.1875
.0123	10	0	0	9.765625E-02
.0081	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .0123 AND .0274 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.664281E-02

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

\*\*\*\*\*

**SUMMARY OF FISHERS EXACT TESTS**

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
1	CONTROL	10	0	
2	0.0081	10	0	
3	0.0123	10	0	
4	0.0182	10	7	*
	0.0274	10	10	*